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Patent application number *(The Patent Office will fill in his part)*	0220211.7
Full name, address and postcode of the or of each applicant *(underline all surnames)*	Glaxo Group Limited Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN, Great Britain
Patents ADP number *(if you know it)*	473587003.
If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom
4. Title of the invention	Vaccine
5. Name of your agent *(if you have one)*	Corporate Intellectual Property
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	GlaxoSmithKline Corporate Intellectual Property (CN9 25.1) 980 Great West Road BRENTFORD Middlesex TW8 9GS
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Vaccine

The present invention relates to novel vaccines for the treatment of asthma or COPD. The vaccines of the present invention comprise an IL-13 immunogen and an adjuvant composition which is (a) a combination of a saponin and a non-toxic derivative of LPS, (b) an immunostimulatory oligonucleotide containing at least one unmethylated CG motif adsorbed to aluminium hydroxide, or (c) a combination of an immunostimulatory oligonucleotide containing at least one unmethylated CG motif and a saponin.

Asthma is a chronic lung disease, caused by inflammation of the lower airways and is characterised by recurrent breathing problems. Airways of patients are sensitive and swollen or inflamed to some degree all the time, even when there are no symptoms. Inflammation results in narrowing of the airways and reduces the flow of air in and out of the lungs, making breathing difficult and leading to wheezing, chest tightness and coughing. Asthma is triggered by super-sensitivity towards allergens (e.g. dust mites, pollens, moulds), irritants (e.g. smoke, fumes, strong odours), respiratory infections, exercise and dry weather. The triggers irritate the airways and the lining of the airways swell to become even more inflamed, mucus then clogs up the airways and the muscles around the airways tighten up until breathing becomes difficult and stressful and asthma symptoms appear.

COPD is an umbrella term to describe diseases of the respiratory tract, which shows similar symptoms to asthma and is treated with the same drugs. COPD is characterised by a chronic, progressive and largely irreversible airflow obstruction. The contribution of the individual to the course of the disease is unknown, but smoking cigarettes is thought to cause 90% of the cases. Symptoms include coughing, chronic bronchitis, breathlessness and respiratory injections. Ultimately the disease will lead to severe disability and death.

A number of recent papers have defined a role for the Th2 cytokine IL-13 in driving pathology in the ovalbumin model of allergenic asthma (Wills-Karp et al, 1998; Grunig et al, 1998). In complementary experiments, lung IL-13 levels were raised by over-expression in a transgenic mouse or by installation of protein into the trachea in wild-type mice. In both

settings, airway hyper-responsiveness, eosinophil invasion and increased mucus production were seen (Zhu et al, 1999).

The amino acid sequence of the mature form of human IL-13 is provided in SEQ ID No. 1.

G P V P P S T A L R E L I E E L V N I T Q N Q K A P L C N G S M V W S I N L T A G M Y C A A L E S L I N V S G C S A I E K T Q R M L G G F C P H K V S A G Q F S S L H V R D T K I E V A Q F V K D L L L H L K K L F R E G R F N *

WO 00/65058 describes a method of downregulating the function of the cytokine IL-5, and its use in the treatment of asthma. In this study, the IL-5 sequence was modified by a number of techniques to render it immunogenic, amongst which there is described an IL-5 immunogen supplemented with foreign T-cell epitopes, whilst maintaining the IL-5 B cell epitopes.

Current treatments of chronic asthma and COPD require frequent and regular administration of therapeutic drugs, which in the case of short acting beta2 agonists can be required several times per day. There is a need for improved treatment methods which do not require such frequent administrations.

The present invention provides novel vaccine formulations for the treatment of asthma or COPD comprising an immunogen that is capable of generating an immune response in a vaccinee against self IL-13 and an adjuvant selected from the group consisting of: (a) a combination of a saponin and a non-toxic derivative of LPS, (b) an immunostimulatory oligonucleotide containing at least one unmethylated CG motif adsorbed to aluminium hydroxide, and (c) a combination of an immunostimulatory oligonucleotide containing at least one unmethylated CG motif and a saponin.

Use of the vaccines in medicine is provided by the present invention. The vaccines of the present invention are used in the manufacture of medicaments for the treatment of asthma or

COPD, and use in novel methods of treatment of asthma or COPD. Also provided by the present invention are methods of manufacturing vaccines of the present invention.

In all aspects of the present invention there is an immunogen that is capable of generating an immune response in a vaccinee against self IL-13. In the case of a human asthma vaccine the immunogen is any immunogen that is capable, when formulated in vaccines of the present invention, of generating an anti-human IL-13 immune response. Preferably the immune response is an antibody response, and most preferably an IL-13 neutralising antibody response that neutralises the biological effects of IL-13 in asthma disease.

As the vaccines of the present invention are to raise an immune response against a self-protein the immunogens of the present invention preferable comprise human IL-13, or immunogenic fragment thereof, which has been rendered immunogenic in a "self" situation (that is to say for use in vaccination of a human with a human protein sequence as the immunogen). A number of techniques have been designed with the aim of breaking "tolerance" to self antigen and thereby rendering them immunogenic in a "self" situation.

One technique involves chemically cross-linking the self-protein (or peptides derived from it) to a highly immunogenic carrier protein, such as keyhole limpet haemocyanin ("Antibodies: A laboratory manual" Harlow, E and Lane D. 1988. Cold Spring Harbor Press).

A variant on the carrier protein technique involves the construction of a gene encoding a fusion protein comprising both carrier protein (for example hepatitis B core protein) and self-protein (The core antigen of hepatitis B virus as a carrier for immunogenic peptides", Biological Chemistry. 380(3):277-83, 1999). The fusion gene may be administered directly as part of a nucleic acid vaccine. Alternatively, it may be expressed in a suitable host cell *in vitro*, the gene product purified and then delivered as a conventional vaccine, with or without an adjuvant.

Another approach has been described by Dalum and colleagues wherein a single class II MHC-restricted epitope is inserted into the target molecule. They demonstrated the use of

this method to induce antibodies to ubiquitin (Dalum et al, 1996, *J Immunol* 157:4796-4804; Dalum et al, 1997, *Mol Immunol* 34:1113-1120) and the cytokine TNF (Dalum et al, 1999, *Nature Biotech* 17:666-669). As a result, all T cell help must arise either from this single epitope or from junctional sequences. Such an approach is also described in EP 0 752 886 B1, WO 95/05849, and WO 00/65058.

Accordingly the immunogens for use in the vaccines of the present invention may comprise modified human IL-13 immunogens, wherein the human IL-13 sequence is modified to include foreign T-cell helper epitopes. The T-cell helper epitopes are preferably "foreign" with respect to human proteins.

Preferably the T-cell helper epitopes are small and are added to the IL-13 sequence by an addition or substitution event within or at the terminal ends of the IL-13 sequence by synthetic, recombinant or molecular biological means. Alternatively the T-cell helper epitopes may be added via chemical coupling of the IL-13 polypeptide to a carrier protein comprising the T-cell helper epitopes. The IL-13 sequences, or functionally equivalent fragments thereof, may also be associated with the T-cell helper epitopes in a fusion protein, wherein the two are recombinantly manufactured together, for example a Hepatitis B core protein incorporating IL-13 sequences.

In the aspects of the present invention where small T-cell helper epitopes are used, a "foreign T-cell helper epitope" or "T-cell epitope" is a peptide which is able to bind to an MHC molecule and stimulates T-cells in an animal species. Preferred foreign T-cell epitopes are promiscuous epitopes, ie. epitopes that bind to a substantial fraction of MHC class II molecules in an animal species or population (Panina-Bordignon et al, *Eur.J.Immunol.* 1989, 19:2237-2242; Reece et al, *J.Immunol.* 1993, 151:6175-6184).
In order for the immunogens of the present invention to be sufficiently clinically effective, it may be necessary to include several foreign T-cell epitopes. Promiscuous epitopes for use in the immunogens of the present invention can be naturally occurring human T-cell epitopes such as those from tetanus toxoid (e.g. the P2 and P30 epitopes, diphtheria toxoid, influenza

virus haemagluttinin (HA), and P.falciparum CS antigen. The most preferred T-cell epitopes for use in the present invention are P2 and P30 from tetanus toxoid.

A number of promiscuous T-cell epitopes have been described in the literature, including: WO 98/23635; Southwood et al., 1998, J. Immunol., 160: 3363-3373; Sinigaglia et al., 1988, Nature, 336: 778-780; Rammensee et al., 1995, Immunogenetics, 41: 4, 178-228; Chicz et al., 1993, J. Exp. Med., 178:27-47; Hammer et al., 1993, Cell 74:197-203; and Falk et al., 1994, Immunogenetics, 39: 230-242. The promiscuous T-cell epitope can also be an artificial sequence such as "PADRE" (WO 95/07707).

The foreign T-cell epitope is preferably selected from the group of epitopes that will bind to a number of individuals expressing more than one MHC II molecules in humans. For example, epitopes that are specifically contemplated are P2 and P30 epitopes from tetanus toxoid, Panina – Bordignon Eur. J. Immunol 19 (12), 2237 (1989). In a preferred embodiment the heterologous T-cell epitope is P2 or P30 from Tetanus toxin.

The P2 epitope has the sequence QYIKANSKFIGITE and corresponds to amino acids 830-843 of the Tetanus toxin.

The P30 epitope (residues 947-967 of Tetanus Toxin) has the sequence FNNFTVSFWLRVPKVSASHLE. The FNNFTV sequence may optionally be deleted.
Other universal T epitopes can be derived from the circumsporozoite protein from Plasmodium falciparum – in particular the region 378-398 having the sequence DIEKKIAKMEKASSVFNVVNS (Alexander J, (1994) Immunity 1 (9), p 751-761). Another epitope is derived from Measles virus fusion protein at residue 288-302 having the sequence LSEIKGVIVHRLEGV (Partidos CD, 1990, J. Gen. Virol 71(9) 2099-2105).
Yet another epitope is derived from hepatitis B virus surface antigen, in particular amino acids, having the sequence FFLLTRILTIPQSLD.

Another set of epitopes is derived from diphteria toxin. Four of these peptides (amino acids 271-290, 321-340, 331-350, 351-370) map within the T domain of fragment B of the toxin, and the remaining 2 map in the R domain (411-430, 431-450):

PVFAGANYAAWAVNVAQVI
VHHNTEEIVAQSIALSSLMV

QSIALSSLMVAQAIPLVGEL
VDIGFAAYNFVESII NLFQV
QGESGHDIKITAENTPLPIA
GVLLPTIPGKLDVNKSKTHI
(Raju R., Navaneetham D., Okita D., Diethelm-Okita B., McCormick D., Conti-Fine B. M. (1995) Eur. J. Immunol. 25: 3207-14.)

The methods of treatment of the present invention provide a method of treatment of asthma comprising one or more of the following clinical effects:

1. A reduction in airway hyper-responsiveness (AHR)
2. A reduction in mucus hyper-secretion and goblet cell metaplasia
3. A reduction in sub-epithelial fibrosis of the airways
4. A reduction in eosinophil levels
5. A reduction in the requirement for the use of inhaled corticosteroids (ICS) would also be a feature of successfull treatment using an IL13 autovaccine. An ideal IL13 autovaccine could potentially make ICS steroid treatment redundant, although a reduction in the 'frequency of use' or 'dose required' of ICS is also envisaged as a valuable outcome.

In the aspect of the present invention where native self IL-13 is coupled to a T-helper epitope bearing immunogenic carrier, the conjugation can be carried out in a manner well known in the art. Thus, for example, for direct covalent coupling it is possible to utilise a carbodiimide, glutaraldehyde or (N-[γ-maleimidobutyryloxy] succinimide ester, utilising common commercially available heterobifunctional linkers such as CDAP and SPDP (using manufacturers instructions). After the coupling reaction, the immunogen can easily be isolated and purified by means of a dialysis method, a gel filtration method, a fractionation method etc.

The types of carriers used in the immunogens of the present invention will be readily known to the man skilled in the art. A non-exhaustive list of carriers which may be used in the present invention include: Keyhole limpet Haemocyanin (KLH), serum albumins such as bovine serum albumin (BSA), inactivated bacterial toxins such as tetanus or diptheria toxins

(TT and DT), or recombinant fragments thereof (for example, Domain 1 of Fragment C of TT, or the translocation domain of DT), or the purified protein derivative of tuberculin (PPD). Alternatively the IL-13 may be directly conjugated to liposome carriers, which may additionally comprise immunogens capable of providing T-cell help. Preferably the ratio of IL-13 to carrer molecules is in the order of 1:1 to 20:1, and preferably each carrier should carry between 3-15 IL-13 molecules.

In an embodiment of the invention a preferred carrier is Protein D from *Haemophilus influenzae* (EP 0 594 610 B1). Protein D is an IgD–binding protein from *Haemophilus influenzae* and has been patented by Forsgren (WO 91/18926, granted EP 0 594 610 B1). In some circumstances, for example in recombinant immunogen expression systems it may be desirable to use fragments of protein D, for example Protein D 1/3rd (comprising the N-terminal 100-110 amino acids of protein D (GB 9717953.5)).

Another preferred method of presenting the IL-13, or immunogenic fragments thereof, is in the context of a recombinant fusion molecule. For example, EP 0 421 635 B describes the use of chimaeric hepadnavirus core antigen particles to present foreign peptide sequences in a virus-like particle. As such, immunogens of the present invention may comprise IL-13 presented in chimaeric particles consisting of hepatitis B core antigen. Additionally, the recombinant fusion proteins may comprise IL-13 and a carrier protein, such as NS1 of the influenza virus. For any recombinantly expressed protein which forms part of the present invention, the nucleic acid which encodes said immunogen also forms an aspect of the present invention.

The following provides preferred specific immunogens.

SEQ ID No 3 is a human IL-13 with P30 inserted into the protein (substituted for the looped region between alpha helices C and D of human IL13) this is an example of a human version of an IL13 autovaccine.

G P V P P S T A L R E L I E E L V N I T Q N Q K A P L C
N G S M V W S I N L T A G M Y C A A L E S L I N V S G C
S A I E K T Q R M L G G F C P H K *F N N F T V S F W L R*
V P K V S A S H L E D T K I E V A Q F V K D L L L H L K
K L F R E G R F N

In an alternative embodiment of the present invention the immunogens comprise foreign – helper epitopes and have a chimaeric IL-13 sequence. In this sense, in the case of a human IL-13 vaccine, the IL-13 immunogen will be based on an orthologous IL-13 sequence (such as the murine IL-13 sequence) wherein at least one, and preferably all, of the murine B-cell epitopes (surface exposed regions) are substituted for the equivalent human sequences SEQ ID NO.14

G P V P P S T A L K E L I E E L S N I T Q N Q K A P L C
N G S M V W S I N L T A G M F C V A L D S L I N V S G C
S A I Y R T Q R I L H G F C P H K V S A G Q F S S L H
V R D T K I E V A H F I T K L L L H L K K L F R E G R F
N

In a related embodiment the chimaeric IL-13 sequence is supplemented with a promiscuous T-cell epitope (P30) added either at the N-terminus of the chimaeric polypeptide. SEQ ID NO. 15

F N N F T V S F W L R V P K V S A S H L E
G P V P P S T A L K E L I E E L S N I T Q
N Q K A P L C N G S M V W S I N L T A G M
F C V A L D S L I N V S G C S A I Y R T Q
R I L H G F C P H K V S A G Q F S S L H
V R D T K I E V A H F I T K L L L H L K K
L F R E G R F N

SEQ ID NO. 16 is a human IL-13 sequence with a promiscuous T-cell epitope added within the IL-13 sequence, substituted into the C-D loop)

G P V P P S T A L R E L I E E L V N I T Q N Q K A P L C N G S M V
W S I N L T A G M Y C A A L E S L I N V S G C S A I E K T Q R M L
G G F C P H K F N N F T V S F W L R V P K V S A S H L E D T K I E
V A Q F V K D L L L H L K K L F R E G R F N *

In certain aspects of the present invention immunogenic fragments of the native IL-13 sequence may be used, for example in the presentation of immunogenic peptides in Hepatitis

B core particles or presentation of human B cell IL-13 epitopes in a non-human IL-13 backbone carrier. In these contexts immunogenic fragments of the human IL-13 sequences preferably contain the B-cell epitopes in the human IL-13 sequence, and preferably at least one, or more, of the following short sequences:

GPVPPSTA
ITQNQKAPLCNGSMVWSINLTAGM
INVSGCS
FCPHKVSAGQFSSLHVRDT
LHLKKLFREGRFN

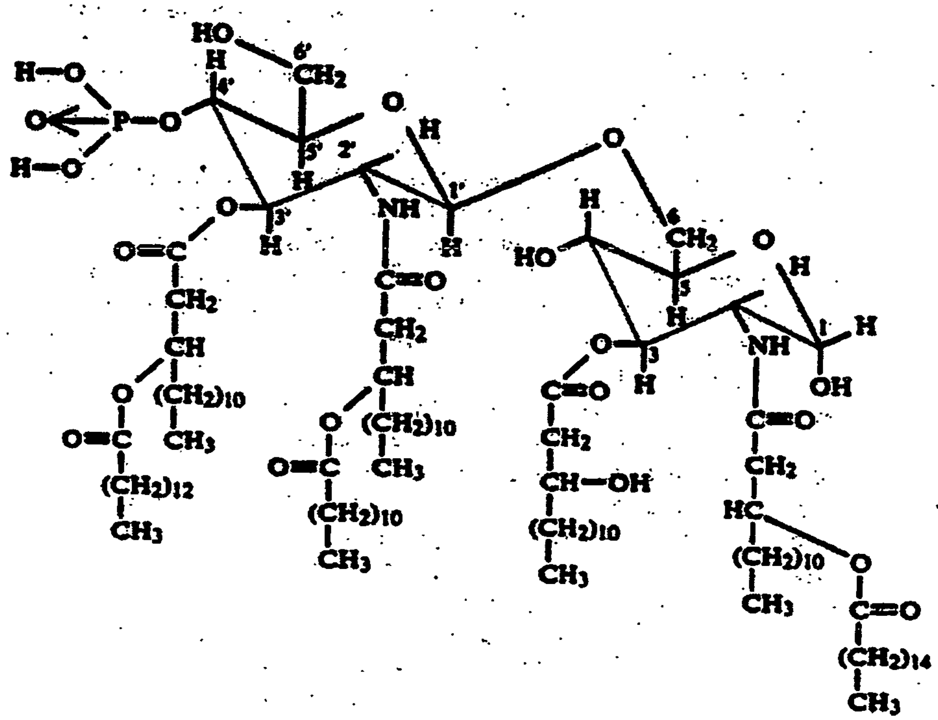
The immunogens as described above form vaccines of the present invention when they are formulated with adjuvants or adjuvant combinations selected from the group consisting of (a) a combination of a saponin and a non-toxic derivative of LPS, (b) an immunostimulatory oligonucleotide containing at least one unmethylated CG motif adsorbed to aluminium hydroxide, or (c) a combination of an immunostimulatory oligonucleotide containing at least one unmethylated CG motif and a saponin.

Saponins are taught in: Lacaille-Dubois, M and Wagner H. (1996. A review of the biological and pharmacological activities of saponins. Phytomedicine vol 2 pp 363-386). Saponins are steroid or triterpene glycosides widely distributed in the plant and marine animal kingdoms. Saponins are noted for forming colloidal solutions in water which foam on shaking, and for precipitating cholesterol. When saponins are near cell membranes they create pore-like structures in the membrane which cause the membrane to burst. Haemolysis of erythrocytes is an example of this phenomenon, which is a property of certain, but not all, saponins.

Saponins are known as adjuvants in vaccines for systemic administration. The adjuvant and haemolytic activity of individual saponins has been extensively studied in the art (Lacaille-Dubois and Wagner, *supra*). For example, Quil A (derived from the bark of the South American tree Quillaja Saponaria Molina), and fractions thereof, are described in US 5,057,540 and "Saponins as vaccine adjuvants", Kensil, C. R., *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1-2):1-55; and EP 0 362 279 B1. Particulate structures, termed Immune

Stimulating Complexes (ISCOMS), comprising Quil A or fractions thereof, have been used in the manufacture of vaccines (Morein, B., EP 0 109 942 B1; WO 96/11711; WO 96/33739). The saponins QS21 and QS17 (HPLC purified fractions of Quil A) have been described as potent systemic adjuvants, and the method of their production is disclosed in US Patent No.5,057,540 and EP 0 362 279 B1. Other saponins which have been used in systemic vaccination studies include those derived from other plant species such as Gypsophila and Saponaria (Bomford *et al.*, Vaccine, 10(9):572-577, 1992).

It has long been known that enterobacterial lipopolysaccharide (LPS) is a potent stimulator of the immune system, although its use in adjuvants has been curtailed by its toxic effects. A non-toxic derivative of LPS, monophosphoryl lipid A (MPL), produced by removal of the core carbohydrate group and the phosphate from the reducing-end glucosamine, has been described by Ribi et al (1986, Immunology and Immunopharmacology of bacterial endotoxins, Plenum Publ. Corp., NY, p407-419) and has the following structure:



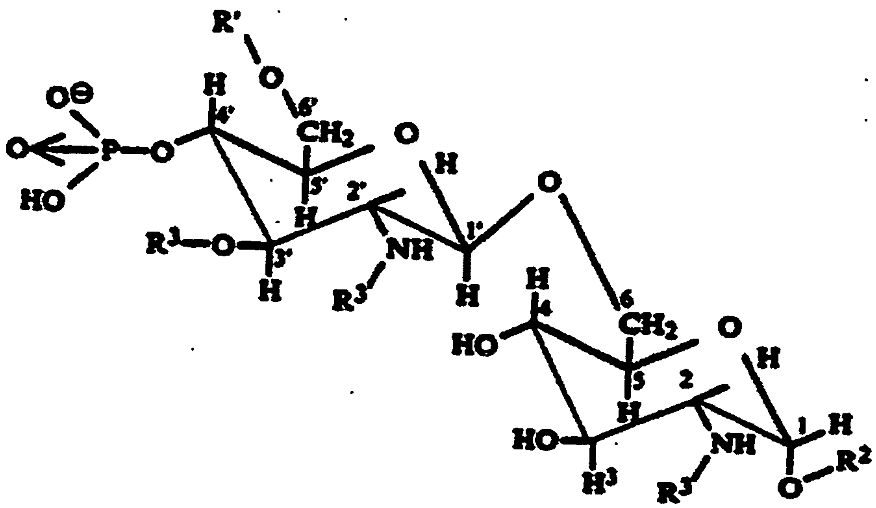
A further detoxified version of MPL results from the removal of the acyl chain from the 3-position of the disaccharide backbone, and is called 3-O-Deacylated monophosphoryl lipid A (3D-MPL). It can be purified and prepared by the methods taught in GB 2122204B, which

reference also discloses the preparation of diphosphoryl lipid A, and 3-O-deacylated variants thereof. A preferred form of 3D-MPL is in the form of an emulsion having a small particle size less than 0.2μm in diameter, and its method of manufacture is disclosed in WO 94/21292. Aqueous formulations comprising monophosphoryl lipid A and a surfactant have been described in WO9843670A2. Other purified and synthetic non-toxic derivatives of LPS have been described (US 6,005,099 and EP 0 729 473 B1; Hilgers *et al.*, 1986, *Int.Arch.Allergy.Immunol.*, 79(4):392-6; Hilgers *et al.*, 1987, Immunology, 60(1):141-6; and EP 0 549 074 B1).

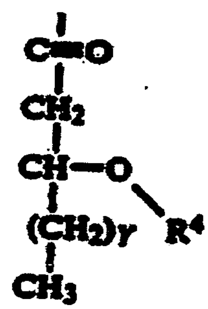
The non-toxic derivatives of LPS, or bacterial lipopolysaccharides, to be formulated in the adjuvant combinations of the present invention may be purified and processed from bacterial sources, or alternatively they may be synthetic. For example, purified monophosphoryl lipid A is described in Ribi et al 1986 (supra), and 3-O-Deacylated monophosphoryl or diphosphoryl lipid A derived from *Salmonella sp.* is described in GB 2220211 and US 4912094. Other purified and synthetic lipopolysaccharides have been described (US 6,005,099 and EP 0 729 473 B1; Hilgers *et al.*, 1986, *Int.Arch.Allergy.Immunol.*, 79(4):392-6; Hilgers *et al.*, 1987, Immunology, 60(1):141-6; and EP 0 549 074 B1). Particularly preferred bacterial lipopolysaccharide adjuvants are 3D-MPL and the β(1-6) glucosamine disaccharides described in US 6,005,099 and EP 0 729 473 B1.

Accordingly, the LPS derivatives that may be used in the present invention are those immunostimulants that are similar in structure to that of LPS or MPL or 3D-MPL. In another aspect of the present invention the LPS derivatives may be an acylated monosaccharide, which is a sub-portion to the above structure of MPL.

A preferred disaccharide adjuvant, is a purified or synthetic lipid A of the following formula:



wherein R2 may be H or PO3H2; R3 may be an acyl chain or β-hydroxymyristoyl or a 3-acyloxyacyl residue having the formula:



wherein $R^4 = -\overset{\displaystyle O}{\overset{\|}{C}}-(CH_2)_X-CH_3$,

and wherein X and Y have a value of from 0 up to about 20.

A yet further non-toxic derivative of LPS, which shares little structural homology with LPS and is purely synthetic is that described in WO 00/00462, the contents of which are fully incorporated herein by reference.

Immunostimulatory oligonucleotides containing at least one unmethylated CG motif are well known adjuvants, and are disclosed in (WO96102555). Typical immunostimulatory oligonucleotides will be between 8-100 bases in length and comprises the general formula X_1 CpGX_2 where X_1 and X_2 are nucleotide bases, and the C and G are unmethylated.

The preferred oligonucleotides for use in vaccines of the present invention preferably contain two or more dinucleotide CpG motifs preferably separated by at least three, more preferably at least six or more nucleotides. The oligonucleotides of the present invention are typically deoxynucleotides. In a preferred embodiment the internucleotide in the oligonucleotide is phosphorodithioate, or more preferably a phosphorothioate bond, although phosphodiester and other internucleotide bonds are within the scope of the invention including oligonucleotides with mixed internucleotide linkages. e.g. mixed phosphorothioate/phophodiesters. Other internucleotide bonds which stabilise the oligonucleotide may be used. Methods for producing phosphorothioate oligonucleotides or phosphorodithioate are described in US5,666,153, US5,278,302 and WO95/26204.

Examples of preferred oligonucleotides have the following sequences. The sequences preferably contain phosphorothioate modified internucleotide linkages.

OLIGO 1: TCC ATG ACG TTC CTG ACG TT (CpG 1826)
OLIGO 2: TCT CCC AGC GTG CGC CAT (CpG 1758)
OLIGO 3: ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG
OLIGO 4: TCG TCG TTT TGT CGT TTT GTC GTT (CpG 2006)
OLIGO 5: TCC ATG ACG TTC CTG ATG CT (CpG 1668)

Alternative CpG oligonucleotides may comprise the preferred sequences above in that they have inconsequential deletions or additions thereto.

The CpG oligonucleotides utilised in the present invention may be synthesized by any method known in the art (eg EP 468520). Conveniently, such oligonucleotides may be synthesized utilising an automated synthesizer. An adjuvant formulation containing CpG oligonucleotide can be purchased from Qiagen under the trade name "ImmunEasy". A

murine adjuvant containing OLIGO 1 and aluminium hydroxide is described in Weeratna *et al.*, Vaccine, 2000, 18, 1755-1762.

The specific adjuvant formulations which may be combined with the IL-13 immunogen to for vaccines of the present invention are selected from the following group:

(a) a combination of a saponin and a non-toxic derivative of LPS,

The preferred adjuvants within this subgroup preferably comprise the saponin QS21, and the non-toxic derivative of LPS which is 3D-MPL.

The QS21 and 3D-MPL can be simply admixed with the IL-13 immunogen (EP 0671 948 B, the entire contents of which are fully incorporated herein by reference), but preferably the adjuvants further comprise a carrier system. The QS21 is preferably associated with a sterol, such as cholesterol, containing liposome, whilst the 3D-MPL can either be associated within the liposome membrane or outside the liposome membrane (as described in EP 0 822 831 B, the entire contents of which are fully incorporated herein by reference)

The QS21 and 3D-MPL can also be associated with an oil in water emulsion comprising a metabolisable oil (WO 95/17210), with or without the presence of a sterol (WO 99/12565, the entire contents of which are fully incorporated herein by reference), and preferably at a low ratio of oil to QS21 (WO 00/11241). The entire contents of WO 95/17210, WO 99/12565 and WO 00/11241 are fully incorporated herein by reference.

The combination of a saponin and a non-toxic derivative of LPS may optionally further comprise an immunostimulatory oligonucleotide containing at least one unmethylated CG motif.

Most preferred adjuvants in this subgroup comprise a mixture of small unilamellar dioleoyl phosphatidyl choline liposomes comprising cholesterol and QS21 at a cholesterol:QS21 ratio of at least 1:1 w/w and preferably with excess cholesterol; and 3D-MPL in aqueous suspension; optionally further comprising an immunostimulatory oligonucleotide in aqueous suspension.

Another preferred adjuvant comprises an oil in water emulsion comprising an aqueous phase and an oil phase, wherein the oil phase comprises oil droplets of squalene and alpha-

tocopherol and a stabilising detergent; optionally further comprising cholesterol; and the aqueous phase comprises QS21 and 3D-MPL, and optionally further comprising an immunostimulatory oligonucleotide.

(b) an immunostimulatory oligonucleotide containing at least one unmethylated CG motif adsorbed to aluminium hydroxide,
The preferred immunostimulatory oligonucleotides are described in OLIGOs 1, 2, 3, 4, or 5; the most preferred oligonucleotide is OLIGO 4. The immunostimulatory oligonucleotides and immunogen are adsorbed onto aluminium hydroxide, and preferably the immunogen and oligonucleotide are adsorbed onto different particles of aluminium hydroxide (WO 00/23105, the entire contents of which are fully incorporated herein by reference). Most preferably the weight:weight ratio's of immunostimulatory oligonucleotide: aluminium hydroxide is approximately 1:1, and preferably between 0.5:1 to 1:0.5.

or (c) a combination of an immunostimulatory oligonucleotide containing at least one unmethylated CG motif and a saponin.
Preferably the adjuvant contains a combination of CpG and saponin as described in WO 00/62800, the entire contents of which are fully incorporated herein by reference. Such adjuvant compositions are also described in WO 00/09159. The most preferred adjuvant combinations of this subgroup comprise QS21 and OLIGO 4. Most preferably the saponin, preferably QS21, is associated with cholesterol containing liposomes, and the, immunostimulatory oligonucleotide, preferably OLIGO 4, is in aqueous solution. Alternatively, the QS21 and immunostimulatory oligonucleotide is presented in an oil in water emulsion, wherein the oil droplets comprise squalene and alpha-tocopherol and a stabilising detergent; the oil droplets optionally further comprising cholesterol (WO 99/12565).

In a related aspect of the present invention there are provided animal pharmaceutical products, for example canine or other veterinary species pharmaceutical products containing autologous IL-13 sequences.

For example, a murine IL-13 immunogen can be manufactured containing the human IL-13 sequence as a backbone, into which the murine IL-13 B-cell epitopes are substituted in place of the human B-cell epitopes.

In a protein vaccine, the amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μg of protein, preferably 1-500 μg, preferably 1-100μg, most preferably 1 to 50μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in vaccinated subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced. Such a vaccine formulation may be either a priming or boosting vaccination regime; be administered systemically, for example *via* the transdermal, subcutaneous or intramuscular routes or applied to a mucosal surface via, for example, intra nasal or oral routes.

There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

It is possible for the vaccine composition to be administered on a once off basis or to be administered repeatedly, for example, between 1 and 7 times, preferably between 1 and 4 times, at intervals between about 1 day and about 18 months, preferably one month. This may be optionally followed by dosing at regular intervals of between 1 and 12 months for a period up to the remainder of the patient's life. In an embodiment the patient will receive the antigen in different forms in a prime boost regime. Thus for example an antigen will be first administered as a DNA based vaccine and then subsequently administered as a protein adjuvant base formulation. Once again, however, this treatment regime will be significantly varied depending upon the size and species of animal concerned, the amount of nucleic acid vaccine and / or protein composition administered, the route of administration, the potency and dose of any adjuvant compounds used and other factors which would be apparent to a skilled veterinary or medical practitioner.

Another aspect of the present invention is a combination of an IL-13 immunogen with 3D-MPL adsorbed to aluminium hydroxide.

Examples

1. Design of a vaccine against murine IL-13

IL-13 belongs to the SCOP (Murzin et al, 1995, *J Mol Biol* 247:536-540) defined 4-helical cytokines fold family. Individual members of this fold superfamily are related structurally, but are difficult to align at the sequence level. The 3D structure of IL-13 has not yet been determined, but structures have been generated for a number of other 4-helical cytokines. Protein multiple sequence alignments were generated for IL-13 orthologues, and also for a number of other cytokines exhibiting this fold where the structure of at least one member had been determined (IL-4, GM-CSF, IL-5 and IL-2). Secondary structure predictions were performed for the IL-13 protein multiple sequence alignment using DSC (King and Sternberg, 1996, *Prot Sci* 5:2298-2310), SIMPA96 (Levin, 1997, *Prot Eng* 7:771-776) and Pred2ary (Chandonia and Karplus, 1995, *Prot Sci* 4:275-285). The individual cytokine protein multiple sequence alignments were aligned to each other, using both the sequence information and the structural information (from the known crystal structures and from the secondary structure prediction).

Antigenic sites, specifically B-cell epitopes, were predicted for murine IL-13 using the Cameleon software (Oxford Molecular), and these were mapped onto the IL-4 structure (accession number 1RCB in the Brookhaven database) using the protein multiple sequence alignment to give an idea of where they might be located structurally on IL-13. From this analysis, exposed regions which were potentially both antigenic and involved in receptor binding were selected.

From this model, a chimaeric IL-13 sequence was designed in which the sequence of the predicted antigenic loops was taken from murine IL-13, and the sequence of the predicted

structural (predominantly helical) regions was taken from human IL-13. The purpose of this design was to identify target epitopes from murine IL-13 against which neutralising antibodies might be raised, and to present them on a framework which was structurally similar to the native protein, but yet contained sufficient sequence variation to the native (murine) protein to ensure that one or more CD4 T helper epitopes would be present. The nucleic acid and protein sequences selected for this example of a chimaeric IL-13 vaccine are shown in Figure 1. The underlined sequences correspond to sequences found in the human orthologue. Twelve amino acids were substituted to achieve the sequence in figure 1. It should be understood that the degeneracy of the genetic code allows many possible nucleic acid sequences to encode identical proteins. Furthermore, it will be appreciated that there are other possible chimaeric IL-13 vaccine designs within the scope of the invention, that have other orthologus mutations in non-exposed areas.

1.2 Preparation of chimaeric IL-13

Chimaeric IL-13 (cIL-13) DNA sequence was synthesised from a series of partially overlapping DNA oligonucleotides, with the sequences cIL-13-1 to cIL-13-6 shown in Table 1. These oligos were annealed, and cIL-13 DNA generated by a PCR with the cycle specification of 94^{0}C for 1 minute followed by 25 cycles of 94^{0}C for 30 seconds, 55^{0}C for 1 minute and 72^{0}C for 2minutes. Followed by 72^{0}C for 7 minutes and cooling to 4^{0}C when finished. The reaction product comprised a band of the expected size, 361 base pairs, which was subcloned into the T/A cloning vector pCR2.1 (Invitrogen, Groningen, Netherlands) to generate pCR2.1-cIL-13. A BamH1 and Xho1 cIL-13 digested fragment from pCR2.1-cIL-13 was then subcloned into the BamH1 and Xho1 sites in pGEX4T3 (Amersham Pharmacia, Amersham, Bucks, UK) generating pGEX4T3-cIL-13/1. On sequencing the pGEX4T3-cIL-13/1 construct we discovered an extra 39 base pairs of DNA sequence (derived from the pCR2.1 vector) between the sequence for GST and cIL-13. To correct this, we repeated the PCR for cIL-13 using pGEX4T3-cIL-13/1 and primers cIL-13Fnew and cIL-13R. The PCR product obtained was then cloned back into pGEX4T3 using BamH1 and Xho1 restriction sites, to generate the expression vector pGEX4T3-cIL-13. The sequence of this construct was verified by dideoxy terminator sequencing. This vector encodes a genetic fusion protein

consisting of glutathione-S-transferase and cIL-13 (GST-cIL-13). The two moieties of the protein are linked by a short spacer which contains the recognition site for thrombin. The fusion protein may be readily purified by glutathione sepharose affinity chromatography, and then used directly, or a preparation of free cIL-13 produced by cleavage with thrombin.

Table 1. Oligonucleotides used to construct chimaeric IL-13.

Oligo	Sequence (5'-3')
cIL-13-1R (SEQ ID NO 10)	TGTGATGTTGACCAGCTCCTCAATGAGCTCCCTAAGGGT CAGAGGGAGAGACACAGATCTTGGCACCGGCCC
cIL-13-2F (SEQ ID NO 11)	AGGAGCTGGTCAACATCACACAAGACCAGACTCCCCTG TGCAACGGCAGCATGGTATGGAGTGTGGACCTGGC
cIL-13-3R (SEQ ID NO 12)	GCAATTGGAGATGTTGGTCAGGGATTCCAGGGCTGCAC AGTACCCGCCAGCGGCCAGGTCCACACTCCATAC
cIL-13-4F (SEQ ID NO 13)	TGACCAACATCTCCAATTGCAATGCCATCGAGAAGACC CAGAGGATGCTGGGCGGACTCTGTAACCGCAAGGC
cIL-13-5R (SEQ ID NO 14)	AAACTGGGCCACCTCGATTTTGGTATCGGGGAGGCTGG AGACCGTAGTGGGGGCCTTGCGGTTACAGAGTCC
cIL-13-6F (SEQ ID NO 15)	AAATCGAGGTGGCCCAGTTTGTAAAGGACCTGCTCAGC TACACAAAGCAACTGTTTCGCCACGGCCCCTTC
cIL-13F (SEQ ID NO 16)	CGCGGATTCGGGCCGGTGCCAAGATCTG
cIL-13R (SEQ ID NO 17)	CTCCGCTCGAGTCGACTTAGAAGGGGCCGTGGCGAAA
cIL-13Fnew (SEQ ID NO 18)	CGCGGATCCGGGCCGGTGCCAAGATCTG

The pGEX4T3-cIL-13 expression vector was transformed into E.coli BLR strain (Novagen, supplied by Cambridge Bioscience, Cambridge, UK). Expression of GST-cIL-13 was induced by adding 0.5 mM IPTG to a culture in the logarithmic growth phase for 4hrs at 37^{0}C. The bacteria were then harvested by centrifugation and GST-cIL-13 purified from

them by a method previously described for purification of a similar GST-human IL-13 fusion protein (McKenzie et al, 1993, *Proc Natn Acad Sci* 90:3735-3739).

2. In vitro mouse IL-13 neutralisation bioassay.

To measure the ability of vaccine generated IL-13 antiserum to neutralise the bioactivity of recombinant mouse IL-13 on human TF-1 cells (obtained in-house), 5ng/ml recombinant mouse IL-13 was incubated with various concentrations of sera for 1 hour at 37^{0}C in a 96-well tissue culture plate (Invitrogen). Following this pre-incubation period, TF-1 cells were added. The assay mixture, containing various serum dilutions, recombinant mouse IL-13 and TF-1 cells, was incubated at 37^{0}C for 70 hours in a humidified CO_2 incubator. MTT substrate (Cat. No. G4000, Promega) was added during the final 4 hours of incubation, after which the reaction was stopped with an acid solution to solubilise the metabolised blue formazan product. The absorbance of the solution in each well was read in a 96-well plate reader at 570nm wavelength.

Note that this assay is only able to measure mouse IL-13 neutralisation capacity in serum dilutions greater than or equivalent to 1/100. Serum dilutions less than 1/100 induce non-specific proliferative effects in TF-1 cells.
The capacity of the serum to neutralise mouse IL-13 bioactivity was expressed as, that dilution of serum required to neutralise the bioactivity of a defined amount of mouse IL-13 by 50% (= ND_{50}). The more dilute serum sample required, the more potent the neutralisation capacity.

2.5 Determination of the level of mouse IL-13 neutralisation required for efficacy in the 'ovalbumin challenge' mouse asthma model.

In order to benchmark the required potency of an IL-13 autovaccine for treatment of asthma, mice were treated with various doses of rabbit anti-mouse IL-13 polyclonal antibody (administered passively by intra-peritoneal injection) during ovalbumin challenge, in the

'ovalbumin challenge' mouse asthma model. Model parameters such as airway hyper-responsiveness (AHR), goblet cell metaplasia (GCM) and lung inflammatory cell content were measured at the end of this experiment. Efficacy in this model was correlated to the levels of mouse IL-13 neutralisation achieved in mouse serum. The mouse IL-13 neutralisation bioassay was used to determine the level of mouse IL-13 neutralisation in serum samples.

Treatment group (Dose of passively administered rabbit anti-mouse IL-13 antibody)	**Mouse IL-13 neutralisation capacity** (ND_{50})
Highest dose	1/4100
High dose	1/2670
Mid dose	1/476
Lowest dose	1/207

Treatment groups given the highest three doses of antibody all performed similarly. All of these three groups showed efficacy equivalent to (for AHR) or better than (for GCM) the gold standard treatment (dexamethasone, administered by the intraperitoneal route at 3 x 1.5mg/kg) used in this model. The 'lowest dose' of antibody administered, showed efficacy somewhere between that of dexamethasone and the 'no treatment' positive control groups.

Therefore the level of IL-13 neutralisation achieved in the 'mid dose' treatment group, represents the required potency threshold for an IL-13 autovaccine in this animal model. The potency threshold is defined as the lowest level of IL-13 neutralisation in mouse serum, required to show 100% efficacy in the asthma model (= ED_{100}). 1x ED_{100} is therefore equivalent to an ND_{50} of 1/476.

3. Vaccination studies

Mice are immunised with protein in adjuvant. The primary immunisation will use ~100ug protein, followed by ~50ug for subsequent boost immunisations. Immunisations will be administered on a 4 weekly basis, serum samples will be taken from the mice 2 weeks after each immunisation (in order to monitor the level of anti-mouse IL13 antibodies and the IL13 neutralisation capacity generated in these serum samples).

Claims

1. A vaccine composition for the treatment of asthma or COPD, comprising an immunogen that is capable of generating an immune response in a vaccinee against self IL-13 and an adjuvant selected from the group consisting of: (a) a combination of a saponin and a non-toxic derivative of LPS, (b) an immunostimulatory oligonucleotide containing at least one unmethylated CG motif adsorbed to aluminium hydroxide, and (c) a combination of an immunostimulatory oligonucleotide containing at least one unmethylated CG motif and a saponin.
2. A vaccine as claimed in claim 1 wherein the immunogen is generates an immune response against human IL13.
3. A vaccine as claimed in claim 2 wherein the immunogen comprises human IL-13 supplemented with foreign T-helper epitopes.
4. A vaccine as claimed in claim 2, wherein the immunogen comprises a non-human IL-13 backbone, substituted with human IL-13 B cell epitopes.
5. A vaccine as claimed in claim 1 wherein the saponin is QS21
6. A vaccine as claimed in claim 1 wherein the non-toxic derivative of LPS is 3D-MPL.
7. A vaccine as claimed in claim 1 wherein the immunostimulatory oligonucleotide has the sequence TCG TCG TTT TGT CGT TTT GTC GTT (OLIGO 4).
8. A vaccine as claimed in claim 7 wherein the immunostimulatory oligonucleotide is adsorbed onto aluminium hydroxide.
9. A vaccine as claimed in claim 1 and claim 8 wherein the weight:weight ratio of immunostimulatory oligonucleotide:aluminum hydroxide is from 0.5:1 to 1:0.5.
10. A vaccine as claimed in claim 1 wherein the vaccine comprises a human IL-13 immunogen comprising an orthologous IL-13 sequence, wherein at least one of the orthologous B-cell epitopes are substituted for the equivalent human sequences.

PG4939

Figure 1.

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    GGGCCGGTGCCAAGATCTGTGTCTCTCCCTCTGACCCTTAGGGAGCTCATTGAGGAGCTG
  1 ---------+---------+---------+---------+---------+---------+  60
    G  P  V  P  R  S  V  S  L  P  L  T  L  R  E  L  I  E  E  L

    GTCAACATCACACAAGACCAGACTCCCCTGTGCAACGGCAGCATGGTATGGAGTGTGGAC
 61 ---------+---------+---------+---------+---------+---------+  120
    V  N  I  T  Q  D  Q  T  P  L  C  N  G  S  M  V  W  S  V  D

    CTGGCCGCTGGCGGGTACTGTGCAGCCCTGGAATCCCTGACCAACATCTCCAATTGCAAT
121 ---------+---------+---------+---------+---------+---------+  180
    L  A  A  G  G  Y  C  A  A  L  E  S  L  T  N  I  S  N  C  N

    GCCATCGAGAAGACCCAGAGGATGCTGGGCGGACTCTGTAACCGCAAGGCCCCCACTACG
181 ---------+---------+---------+---------+---------+---------+  240
    A  I  E  K  T  Q  R  M  L  G  G  L  C  N  R  K  A  P  T  T

    GTCTCCAGCCTCCCCGATACCAAAATCGAGGTGGCCCAGTTTGTAAAGGACCTGCTCAGC
241 ---------+---------+---------+---------+---------+---------+  300
    V  S  S  L  P  D  T  K  I  E  V  A  Q  F  V  K  D  L  L  S

    TACACAAAGCAACTGTTTCGCCACGGCCCCTTCTAA
301 ---------+---------+---------+------  336
    Y  T  K  Q  L  F  R  H  G  P  F  *
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PCT Application
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